or hypoxic conditions [3]. Mutations have been introduced into yeast mitochondrial DNA to target residues in hydrophilic regions of Cox1. In addition, a series of mutants was constructed in which specific Cox5 isoforms were expressed or in which residues of Cox5A were mutated to match those in 5B. The ability of all mutant strains to assemble a stable C*c*O and grow on respiratory medium was assessed and their O_2-consumption rates were measured. Selected C*c*Os with mutations in the D and H channels were purified to investigate their effects on C*c*O functioning. Mutation of D channel residues E243D and I67N allowed the definitive assignment of E243 as the essential protonated carboxyl group that appears in CO photolysis [4] and redox FTIR difference spectra of mitochondrial forms of C*c*O. Effects of mutations in the H channel and in Cox5 are discussed in terms of the role of the H channel and the potential of supernumerary subunit isoforms to influence catalysis.

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doi:10.1016/j.bbabio.2012.06.295

14P17

The function of Cox7a1 for brown fat thermogenesis (S14 terminal oxidases)

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Cox7a1 is one of two isoforms of subunit 7a in cytochrome *c* oxidase (COX), which is the terminal respiratory chain enzyme in mammals. This protein is predominantly expressed in tissues with high aerobic capacity and a large number of mitochondria such as heart and skeletal muscle. Cox7a1 protein expression is also present in brown adipose tissue (BAT) — a specialized tissue, providing adaptive non-shivering heat production in order to maintain normothermia in mammals exposed to low environmental temperatures. Importantly, thermogenesis in BAT is mediated by an increased COX activity and elevated levels of uncoupling protein 1 (UCP1), resulting in a shift from coupled towards uncoupled proton translocation in mitochondria. In a previous study we have shown that Cox7a1 protein levels in BAT are strongly increased in cold-exposed (4 °C) mice compared to room-temperature (20–22 °C) acclimated animals [1]. For this reason, we hypothesize that Cox7a1 replaces Cox7a2 in BAT to serve as more active isoform of COX-subunit 7a, thereby increasing COX activity and BAT thermogenic capacity at low ambient temperatures. To study this phenomenon, we employ Cox7a1 knockout mice.

In our study, we compare BAT function in wildtypes and knockouts that were either housed at 5 °C (cold-exposed) for variable periods (4–15 days) or constantly kept at room-temperature (control group). Cox7a1 and Cox7a2 mRNA were detected in wildtypes of all groups. Cold-exposed wildtypes exhibit an increased Cox7a1 to Cox7a2 ratio (>1), indicating BAT to be one of the major sites of Cox7a1 expression. Although cold-exposure leads to elevated COX activity in BAT as expected, differences in the oxygen consumption of complex IV between wildtypes and Cox7a1 knockouts were not detected within the groups. Since measurement of COX activity was so far performed in tissue homogenates, thus measuring the oxygen consumption of solubilized complex IV, upcoming experiments will predominantly focus on BAT function and activity *in vivo* in wildtypes and knockouts.

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doi:10.1016/j.bbabio.2012.06.296

14P18

Electrochemical analysis of cytochrome ba_3 from *T. thermophilus* immobilized on gold nanoparticles

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Protein film voltammetry has been established as a very convenient method for studying electrochemical and catalytic properties of redox enzymes [1]. It is a real challenge, however, to immobilize on the surface of electrodes a large amount of integral membrane proteins and to establish a good electronic communication with the enzyme cofactors while maintaining a suitable environment for these large complex structures.

We are using 3D gold nanoparticle networks as the conductive support of immobilization for membrane proteins and fragments [2]. The gold nanoparticles, indeed, have been shown to mediate the long-range electron transfer between the electrode and the cofactors and their high surface to volume ratio allows at the same time a high coverage of the electrode with proteins [3].

We will report the electrochemical analysis under aerobic and anaerobic conditions of cytochrome ba_3 from *Thermus thermophilus* [4] immobilized on gold nanoparticles. The influence of the pH will be discussed. The structure of the immobilized membrane proteins will be carefully monitored by Surface-Enhanced vibrational spectroscopies, both IR (SEIRAS) and Raman (SERRS). The studies will be completed by FTIR difference spectroscopy to show the influence of the heme propionates in the pH dependence.

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doi:10.1016/j.bbabio.2012.06.297

14P19

Study on the catalytic current in the cytochrome *c* oxidase from *P. denitrificans*

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Cytochrome *c* oxidase couples the reduction of oxygen to water to the translocation of 4 protons across the membrane. Oxygen reduction occurs at a binuclear center composed of a heme a_3 and a Cu_B center. During oxygen reduction, chemical protons access the binuclear center through 2 proton pathways, namely the K- and the D-pathway. These pathways form a hydrogen bonding network facilitating proton diffusion [1]. Residues crucial for proton translocation have previously been reported by biochemical, structural and spectroscopic techniques [2,3].

We have developed an electrochemical approach that allows studying the electrochemical and catalytic properties of redox active membrane proteins. Efficient electron transfer is possible due to the immobilization of the membrane proteins on gold nanoparticle networks which provide a high surface to volume ratio and consequently allow to immobilize a significant amount of protein on the electrode surface [4,5]. These particles also act as a relay in the long-range electron transfer between the electron and the cofactors.

The catalytic current of wild type cytochrome *c* oxidase from *Paracoccus denitrificans* and of variants with mutations introduced into crucial positions of the D- and K-pathway was determined. Interestingly some activity remained even for the mutant enzymes that are reported to be inactive. These results are presented together with the effect of Zn inhibition.

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doi:10.1016/j.bbabio.2012.06.298

14P20

Cytochrome *c* oxidase heme and Cu centres: Redox and spectral interactions

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In beef heart cytochrome *c* oxidase turning over aerobically with ascorbate and cytochrome *c*, the three redox centres heme *c*, heme *a* and CuA are at near equilibrium in fully active and partially inhibited states [1]. In the presence of formate there is no reduction at the binuclear centre (heme a_3/CuB). E_0' values (pH 7.4, 30 °C) are +310 mV for heme *a* and +260 mV for CuA, if E_0' cyt. *c* is +255 mV. This standard redox potential difference between heme *a* and CuA permits determination of their separate difference spectra. Cupric CuA has positive absorbances in the 500–600 nm region plus the characteristic 835 nm band. The 605 nm reduced heme *a* alpha peak is red-shifted in the presence of oxidized CuA. Heme *a* and CuA titrations are close to simple Nernstian one-electron processes, indicating almost no redox interaction between the centres. But there are both redox and spectral interactions between the binuclear centre and the heme *a*/CuA system. Reduction and/or ligation of the binuclear centre decreases the redox potential of the haem *a* and induces shifts in the heme *a* spectrum. These interactions affect the kinetic analysis of the enzyme as well as the use of the CuA steady state in determining the functional status of the terminal oxidase *in vivo*.

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doi:10.1016/j.bbabio.2012.06.299

14P21

Cytochrome c oxidase signalling impact: Does the phosphorylation status really correspond to the enzyme kinetics or its enzymatic activity?

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Mammalian cytochrome c oxidase (CcO) is a dimeric multifunctional mitochondrial enzyme consisting of 13 subunits in each monomer. The study of kinetics and phosphorylation status of the enzyme provides an insight of its possible regulatory function and shed a light on its role in the network of mitochondrial respiratory chain. Using KinasePhos 2.0 web server, multiple phosphorylation sites are predicted in various subunits of CcO. These predicted sites are shown on the crystal structure of the enzyme with already identified phosphorylation sites. Although by default, several phosphotyrosine sites are predicted but with 90% specificity, no subunit phosphorylation was suggested at the tyrosine residues in the whole enzyme. Interestingly, this is in contrast to the already known findings of Tyr_{304} at I [1], Tyr_{218} at II [2] or Tyr_{11} at IV −1 [3]. Even the Thr_{35} at Va [4] was not predicted by this web server. Therefore, we conclude the dynamic changes in the phosphorylation patterns of CcO corresponding to the metabolic and respiratory status of mitochondria. In order to compare, BN PAGE isolated enzyme was incubated under the same